THE ORGANIZATION AND EARLY DEVELOPMENT OF THE EGG OF CLAVA LEPTOSTYLA AG.

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A recent paper by Harm ('02), on the development of *Clava squamata* describes several features so unlike conditions which I have found in the American species, that it has seemed worth while to study anew, and in some detail, the entire life history, including also some interesting peculiarities of the organization of the egg not hitherto given any degree of attention among cœlenterates.

The general facts concerning the morphology, habits, etc., of *Clava leptostyla* Ag. were described long ago by Professor Agassiz ('62) with sufficient fulness to obviate the necessity of any considerable details as to these points. Allman ('71) has likewise given a somewhat similar account of *C. squamata*, though lacking in embryological details.

The following account is based upon studies carried on at Woods Holl during some two summers, chiefly upon living material, though for the details of cytology specimens were killed by several standard methods, among which were corrosive-acetic acid, picro-acetic, Carnoy's solution, Petrunkevitch solution, and corrosive formalin. Upon the whole, the first two of the reagents named gave by far the better fixation. As will be noted in another connection, for certain cytological results the picro-acetic solution was exceptionally good.

The most common habitat of the species in this vicinity was found to be the fronds of the common rock-weed, *Fucus nodosus*. Occasionally I have taken it from the piles of docks, or similar situations. It occurs most frequently in shallower harbors, or tide-pools, and is frequently found at low tide quite exposed, except as the moist vegetation may afford a measure of protection, lying thus for hours subject to the desiccating influences of sun and air, and apparently without serious injury. Of course, this

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vicissitude is common to many organisms of similar habitat, yet few are so little protected by some skeletal feature as is this wholly naked hydroid. The breeding season at Woods Holl is apparently during June and early July, though colonies may be found in seemingly thriving conditions both earlier and later. Whether *Clava* like *Pennaria*, *Eudendrium* and others, has its phases of decline and recovery I have not been able to determine from actual observation, though the fact that at recurring intervals it appears in the same locations would seem to support this view.

Origin and Growth of the Eggs.

Weismann ('83) was the first to give any direct attention to this problem in *Clava*. According to him the ova arise in the entoderm in close relation to the supporting layer, though suggesting that perhaps the most primitive stages may have an ectodermal origin.

On this point Harm ('02) is quite specific in claiming their origin in the ectoderm, and their later migration into the entoderm, where in the region of the gonophores they become definitely differentiated and complete their development. This author even goes so far as to suggest that they may be distinguished in the planula as primitive germ cells, — "urkeimzellen" (p. 47, Fig. 48).

Of course, I am not prepared to discuss the matter so far as it relates to *C. squamata*, not having seen this species, but so far as it concerns *C. leptostyla* I have no hesitation in saying that eggs probably never arise in the ectoderm, but always in the entoderm of the peduncle of the gonophore, or in that of the polyp very near the base of the gonophore. In thousands of sections studied, both by myself and by several of my students, there has been no exception to this statement of fact. *Clava*, like other hydroids, has its breeding season, during which the germ-cells are extremely abundant, and at other times these cells are either entirely absent or very scarce. Again they do not appear except in direct relation to the forming gonophores or in that immediate region. In fact in *Clava leptostyla* the morphology of the gonophores and their development as dense, bud-like clusters from a single peduncle to which they are attached by narrow pedicels, make it

almost certain that the vast majority of all egg-cells arise in the gonophores themselves as, of course, is the case in almost all free gonophores, or medusæ, and as is invariably the case with the spermatozoa.

Therefore, while it may not be impossible that "urkeimzellen" should perhaps exist in undifferentiated stages, still the probability is so extremely remote as to render doubtful to a degree any but the most thoroughly substantiated claims.

It may be stated in passing, that the gonophores of *C. leptostyla* are extremely degenerate, hardly more, indeed, than sporosacs, yet it is possible to distinguish rudiments of medusoid structures. They originate as buds of the hydroid, involving both ectoderm and entoderm, and also supporting layer. Occasionally this lamella seems to partially disintegrate at the terminal portion during the outgrowth of the peduncle. The gonophores remain entirely closed except at the time of rupture by the escaping planula. From the primary peduncle secondary pedicels arise, forming a racemose-like cluster, within each of which from one to four eggs may develop, though the usual number is two or three.

There seems to be considerable variation in the size of the eggs in various specimens and in the eggs of various gonophores of the same specimen. On this point it had occurred to me that perhaps the number developing in a given gonophore might naturally have some influence, but after comparing a considerable number, whether growing singly or in clusters I have not been able to convince myself that such is the case. I am rather inclined to believe that more depends upon the start a given egg may get in growth, and perhaps the state of nutrition in which the given specimen may be at the time, than any other factors.

As a rule eggs which grow singly at the distal end of a gonophore are more nearly spherical, and in consequence I find the cleavage of such eggs much more regular and symmetrical than in cases where two or more are found in the same gonophore and approach maturity at about the same time. This point may be considered in more detail in a later connection under the subject of cleavage.

Growth takes place, as in most hydroids, quite rapidly. With

the origin of the gonophores the eggs appear in considerable numbers in the entoderm of the peduncle, as previously stated, and with the later budding of the gonophore bodies either migrate into them, or as seems to be the case in many instances, originate directly from the entoderm of the spadix or lateral walls of the gonophore. At first they have the characteristic aspects of ordinary ovocytes, namely, a very large germinal vesicle, with characteristic chromatin network, a comparatively small proportion of cytoplasm, which is more or less homogeneous in texture, and staining quite uniformly with any of the ordinary plasma stains. Evidences of growth are first indicated by the rapid increase in the mass of the cytoplasm, while that of the nucleus for a time remains apparently unchanged, though later also increasing in mass likewise, though to a much less degree. Nutrition of the ova is at first, indeed throughout so far as I can determine, by direct absorption from the entoderm of the spadix, or to a less degree also from the entoderm of the lateral walls of the gonophore. I find no evidence of the absorption of supernumerary ovocytes involved in the matter of nutrition, and in this respect *C. leptostyla* appears to differ somewhat from *C. squamata*. According to Harm there would seem to be involved both these processes. He says that by the direct assimilation of yolk-like granules from the entoderm cells of the hydranth, and by osmosis from the walls of the gonophore the egg is nourished, and that furthermore, the youngish egg-cells are also nourished by the absorption of ovocytes (*op. cit.*, pp. 11, 12).

The presence of the yolk-like particles to which he refers I have also recognized in the entoderm of the hydranth body at this period. They resemble in all essentials the pigmented yolk granules later found in the fully grown egg, but I have found no evidence that they are ever directly absorbed by the young egg. On the other hand there is ample evidence to the effect that they are gradually broken down and probably liquefied, in which condition they may be easily transferred to the gonophores and absorbed by the young eggs. In an earlier paper ('04) I have directed attention to similar phenomena in the growth of the eggs of Pachycordyle, and it undoubtedly occurs in many others.

Hermaphroditism.

An interesting feature in the reproduction of this hydroid is the fact that occasionally individuals, and perhaps colonies, are found in which gonophores contain both eggs and spermatozoa. Figs. 1, 2; Fig. 5, Pl. IX., show various phases of this somewhat anomalous condition. As will be observed, the elements are in various stages of development, some of the eggs well along toward full growth, and spermatozoa likewise well advanced. In most cases the condition shown at Fig. 5, Pl. IX., was the prevalent one, namely, where one half of the gonophore bore sperms and the other half an egg. In a few cases, however, a well developed egg was found on each side nearly or quite surrounded by sperm-cells.

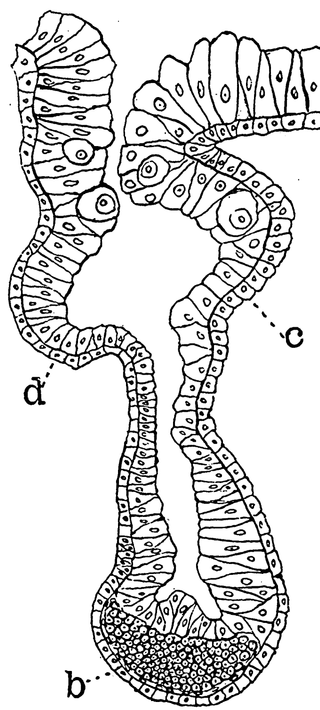


Fig. 1. Longitudinal section through young gonophore, showing at *b* the development of spermary, at *c* and *d* mixed gonads.

Of course, hermaphroditism in itself is nothing strange among animals, whether high or low. Even among hydroids it is quite familiar in the common *Hydra*, though here it is not common to find both organs in active function at the same time on any given individual. I have also found a similar condition in *Hydractinia*, and Bunting ('94), has likewise figured a single case though without giving any details concerning it. In the whole of his extended researches on the "Origin of Sex Cells in Hydromedusæ" Weismann makes no mention, so far as I have observed, concerning hermaphroditism. It would seem somewhat remarkable that he should not have observed some indications of such a condition if it were at all common. Indeed, though having found repeated cases of it in *Clava*, I am disposed to consider it as a rather rare phenomenon in this group.

One other feature in connection with the subject must be noted, namely, that among the several cases, the ova were found in every case in distinctly male gonophores. Among hundreds of female gonophores examined there was not the slightest evidence of male elements among them. Figs. 1 and 2 show sections through two hermaphroditic gonophores. At *b* Fig. 1 is developing a

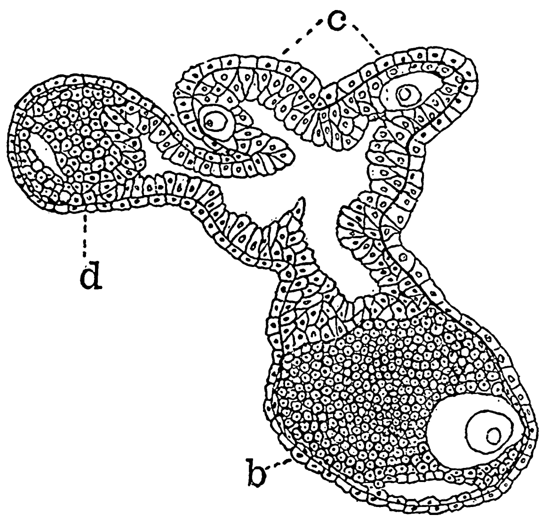


FIG. 2. Cross-section of hydranth showing development of gonophores. *b*, hermaphrodite gonophore ; *c*, early stages of same ; *d*, spermary.

typical male gonad, while at *c* and *d* are shown what are evidently destined to be mixed gonads. Similar conditions are also shown in Fig. 2, which is a camera sketch of a section across the entire hydranth and gonophores.

It has long been known that among actinians a form of hermaphroditism, involving successive sexual rhythms, known as protandry or protogyny, occurs. Duerdon ('04), in his studies of West Indian corals has found warrant for believing that protogyny is the predominant condition, since "spermaries have never been found alone, but always associated with large numbers of ova ; on the other hand, many polyps have been found with ova alone, often few in number, as if sexual maturity were but beginning." At the same time he quotes from Mr. Stanley Gardiner, who having studied a large number of developmental stages in *Flabel-*

lum rubrum, has been able to show that in this form spermaries arise first on the mesenteries and that ova appear later, when the production of sperm acini ceases. "The ova grow enormously with the final result that the mass becomes entirely female, consisting usually of two or three large ova, flattened on their sides against one another and occupying the whole area of the former testes."

Hermaphroditism is also known among Scyphomedusæ, Wright having described in some detail the chief features in the case of *Chrysaora hyoscella*. He says, "Large individuals are hermaphroditic, but smaller ones are found which are unisexual, the male or female element being suppressed. Small Chrysaoras (about four inches in diameter), have no ovarian bands in their pouches, which only contain masses of the grape-like bodies (testes), and tentacles before mentioned."

This would seem to imply that here again we have protandrous hermaphroditism, the spermaries developing first, and later the ovaries. Incidentally, it may be observed in passing, that this author in his account of the development of the eggs of this medusa was much impressed with the absence of any germinal vesicle. "The ova of *Chrysaora hyoscella* do not present, at any stage, a trace of the germinal vesicle, — objects which are so readily detected in the ova of other polypoid Zoophytes."

Haeckel ('79), who has also studied the development of the medusa, was able to confirm Wright's account as to hermaphroditism. "In Uebereinstimmung mit letzteren habe ich gefunden, dass junge Chrysaoren rein männlich vorkommen, solche mittleren Alters meisters Hermaphroditen sind, und endlich ganz alte Thiere meisters nur weiblich sind, oft noch mit Ueberresten männlicher Organe."

So far as I have been able to discern in the case of *Clava*, there is no evidence to indicate the operation of either of these oscillating phases of sexualism. I have specimens taken at all times of the breeding season and have found no tendency toward the one or the other. I am rather disposed to regard it as an expression of a mutative impulse, in response to which in other forms, such as those already cited, these interesting features became established.

Organization of the Egg.

It has long been known that simple though the egg may be it must, nevertheless, be regarded as potentially highly complex. Concerning the early views of His, Whitman, Flemming and other earlier investigators, no attempt will be made here to give special citations. The later experiments of Roux, on the development of the frog's egg, supplemented by similar experiments by Driesch '95, served to emphasize still further the general view here stated.

The still later investigations of Wilson ('03, '04), and Conklin ('05[1], '05[2], '05[3]), have given a new impulse to researches along this line, and have clarified and measurably harmonized the conflicting views of earlier observers.

So far as I am aware, no one has shown any evidence of anything of a similar character in the organization of the eggs of cœlenterates. In commenting on the account given by the present writer of the cleavage of the egg of *Pennaria* Conklin ('05[3]), has suggested the probability that predetermining factors must be present in some form. "Even in such eggs as that of *Pennaria* it is certain there must be determining factors somewhere, if not in the cytoplasm then in the nucleus, which determines that the egg shall develop into a pennaria rather than into some other animal; it is further evident that these determining factors must be present in the cytoplasm at a relatively early stage, if not in the very beginning of development" (p. 215).

Hence in my studies on hydroid eggs rather particular attention was given to this point with the hope that some evidence might be found for or against the views in question. In my work on *Pennaria* ('04[3]), while no direct evidence of cytoplasmic differentiation was found in the living egg, there was found in some cases after fixation by certain reagents, more particularly the picro-sulfuric solutions, what seemed to be a stratified, or concentric arrangement of the cytoplasm. At first I was inclined to regard this as probably significant of such locally differentiated matter as might go to form the ectoderm. But since this condition was not found to be constant it was regarded as probably an artifact, due to the action of the reagent. A recent examination of my earlier preparations has not suggested any change of

this opinion. Indeed, as pointed out before, the extremely erratic and anomalous behavior of these eggs during cleavage would seem to render extremely difficult, if not impossible, any predetermining factors in either cytoplasm or nucleus, whose influence could be maintained during the varying and indeterminate process of development. While we may readily admit that, as Conklin has suggested, there must be factors present which determine that the egg shall develop into a *Pennaria*, still this does not compel the conclusion that therefore they must be definitely localized. It is simply a matter of heredity; and if it be true as generally contended that this is a problem of chromosomes; and if, as I have shown in the case of both *Pennaria* and *Eudendrium*, the chromatin may be more or less dispersed throughout the entire cytoplasm during maturation and early cleavage, then definite localization in one or the other is not involved in Conklin's sense of the term. However, it must be regarded as a question of fact, and so far as evidence exists in the present case it would seem to be opposed to the theory of localization.

During June and July of the past summer I carefully studied the living eggs of *Clava*, and with this point still clearly before me. As pointed out in an earlier section, the eggs originate in the entoderm of the gonophore, and grow by direct nutrition derived from the cells of that tissue. A study of the eggs in various stages of growth revealed the appearance at a certain stage of development of a delicate, bluish pigment, which gradually accumulated in amount as the eggs approached maturity. This was carefully observed in the living specimens and has since been studied in sections after a variety of fixations. At first the pigment makes its appearance in the immediate region of the nucleus, about the time that body takes its place at the outer periphery of the egg. This is shown in Fig. 1, Plate I. From the nuclear region the pigment extends as a crescentic disc outward, forming later a peripheral zone which finally extends over the entire egg, though this rarely occurs until cleavage has made some progress.

An interesting fact observed in this connection was that the amount, or at any rate the color-intensity, of the pigment differed considerably in different specimens. This was particularly the

case in colonies which had been kept in the aquarium for a few days. In these the pigmentation was appreciably less intense than in specimens freshly collected. There were, however, notable exceptions in this respect among various specimens under natural conditions. The same has been observed in *Pennaria*, and is, indeed, a fact more or less well known in many animals. Conklin ('05[1], p. 13) has cited similar cases among ascidians observed by VanBeneden and Julin; in some species two very differently colored eggs being produced, one yellow, the other gray. Both are said to develop normally and in the same manner, giving rise to larvæ whose entoderm cells are of the same respective colors.

In the case of *Clava* there is no such distinction as this, though the presence of more or less pigment has apparently no effect upon the normal development of the embryo. In another point there is also some measure of similarity, namely, in that as development proceeds, the pigment which was at first distinctively peripheral in position, seems later to become transferred to the entoderm of the larva. This, however, as will be seen later, is due not to any shifting of this matter from one region to another, but simply its resorption by the more rapidly developing ectoderm, while as yet the entoderm is only partially differentiated.

Whether this pigmentary zone formed a definite germinal area, and its gradual development was an expression of the differentiation of this area, or whether it might not be simply the results of cytoplasmic metabolism associated with the formation of yolk substance, or whether it might not, perhaps, be in some way associated with the phenomena of maturation, seemed for a time somewhat uncertain. A study of the matter more critically in its cytological aspects soon sufficed to discredit the last alternative, namely, that it was in any way associated with maturation. Furthermore, the appearance of the pigment was too early in the history of the egg to involve the operation of any maturation phenomena.

Again, several series of facts conspired to discredit the probability of the first alternative, namely, that it was in any sense a differentiation of germinal substance. Of these the following may

be particularly mentioned: (1) The entire absence of any correlation between the pigmentary zone and the course of cleavage; (2) the continuous development of pigment, even after the completion of cleavage, and after the ectoderm has been clearly established; (3) the staining reactions of the granules after hardening is comparable, point by point, with that of yolk granules; (4) finally, the granules are gradually reduced and resorbed with the growth of the embryo. It is in this way that their disappearance from the ectoderm above referred to is to be explained.

I think that we may therefore conclude that, at least so far as the problem is concerned with *Clava*, its solution is unquestionably in the negative. If further warrant be needed for this view I believe it will be found in the later history of these pigmentary granules as given in the following account.

Origin and Growth of Pigmentary Granules.

As already noted the origin of these pigmentary granules is in the immediate region of the nucleus, and about the time this body reaches the outer periphery of the egg. At first they are of extremely small size, about 0.5 micra in diameter, later growing and reaching in some cases a diameter of 3 to 3.5 micra. For some time they were entirely overlooked in prepared material, owing to improper fixation. Only after fixation with picro-acetic (or to less degree with Petrunkevitch) solutions and staining with iron-hæmatoxylin on the slide were they adequately differentiated so as to be readily studied. Wilson gives a similar account of this technic in reference to the eggs of *Dentalium* (cf. *Jr. Exp. Zool.*, Vol. I., p. 9, Figs. 10–13, explanations).

As the granules continue to grow larger there may be distinguished within their substance what appear to be vacuoles, usually a single one within each granule, occupying an eccentric position. In some respects they exhibit nucleolar-like features, especially in their staining reactions and in their vacuolation. Montgomery ('98) has made similar observations on nemerteans and believes they indicate some sort of genetic relationships between nucleoli, yolk balls and granules. My own observations have not seemed to confirm this last point, though in the case of *Hydra* there are not lacking evidences which I think would give

strong confirmation of Montgomery's views. This point may have further consideration in connection with observations upon the history of the nucleolus.

Concerning the real nature of these granules there arises the query, are they katabolic products, associated with some vital wastes incident to the cytoplasmic activities of growth, or are they not rather anabolic in character, highly nutritive proteid bodies, analogous to yolk matter and of similar import? The latter is by far the more probable view, though there are points of difference as compared with the usual formation of such nutritive matters. For example, in most hydroid eggs which have come under my observation the development of yolk granules has no appreciable relations to nuclear influence, and seems to be for the most part developed and deposited chiefly at the vegetal pole of the egg; while in the present case, as has been shown, they seem to arise and develop chiefly in the nuclear area and only at a late period are found at the vegetal pole. However, I am inclined to believe that this is not a serious difficulty. It will be observed that the vegetal pole lies in immediate contact with the spadix of the gonophore, and that the reception of nutritive matter by the growing egg is from this source. Of course, this nutritive matter is in the form of liquid, and so long as the egg is continuously receiving it in this way there is no occasion for further metabolism into the more solid reserve of yolk. The remoter animal pole where such reserve would first be needed begins the anabolic process first, and with the gradual suppression of the nutritive activities of the spadix the process of proteid anabolism would extend into that area.

According to Harm (*op. cit.*, p. 19), the development of this yolk matter in *C. squamata* presents some rather sharp contrasts as compared with the above. For example, instead of developing more or less gradually, and spreading from the nuclear region over the surface of the egg, he finds it arising somewhat suddenly and equally throughout the entire egg. "Bald nachdem die Eizellen Glockenkern erreicht haben, beginnt in ihnen die Dotterbildung, die an allen Stellen zu gleicher Zeit einsetz." He also finds that before the formation of yolk granules the eggs are reddish in color. "Während die lebende Eizelle vor der Dotterbil-

dung röthlich erscheint, zeigt nacht derselben eine blaugraue Fährbung." This point is confirmed in the case of *C. leptostyla*, in which essentially the same process takes place.

Perhaps a few words may be added as to the significance of the development of pigment in connection with these yolk granules. I have in recent papers ('04[1], '04[2]), submitted certain views as to this subject and it may suffice in general to refer to those discussions. In the former it was said that pigments in organisms might appear under three aspects: (1) Those directly serviceable to the organism, as in chlorophyll, hæmoglobin, etc.; (2) as waste products, which embrace probably the more numerous of organic pigments, such as guanin, melanin, etc.; (3) as reserve products, of which the lipochromes are typical. In all probability the various pigmentary matters found in eggs belong to the third of these classes. And here undoubtedly should be classed the pigmentary granules of *Clava*, and other similar pigments of hydroid ova. In the second paper attention was directed to a special case, that of *Pachycordyle*, already referred to, in which one may trace the various stages in the growth of the egg and the formation of the pigmentary bodies. Here as in *Clava* there can hardly be reasonable doubt that the process is a gradual and progressive anabolism, so far as the granules themselves are concerned, but it must still be a somewhat open question as to the exact relation of pigmentation thereto. May it not be probable that here, as in many of the more active phases of metabolism in which pigments are more or less evident expressions of excretory, or waste products, the pigment itself, though associated with anabolic activities, is an expression of the correlative process of catabolism? In other words, that even in those constructive processes involved in the storage of reserve matters, whether as proteids, fats, or whatever they be, there is involved the inseparable process of energy bestowed, and that as one of the signs of such energy its imprint is left in these pigmentary elements? Such I am inclined to believe is what actually happens. And as the nature of these processes differ more or less in various organisms so the pigmentary signs of waste will likewise differ. Hence the purple pigment of the eggs of *Clava*, the pinkish of *Pennaria*, the reddish of *Eudendrium*, etc.

Maturation.

Concerning this phase of development there is comparatively little to be said in the case of *Clava.* I have studied as critically as the nature of the egg would allow the behavior involved in maturation both in living and preserved material, and in a very large number of preparations, but with almost wholly negative results. That is, I have found the phenomena to be so obscured by the opacity of the cytoplasm, or by the pigment matter in the yolk, or as seems to me a still further probability, namely, the extremely fugitive character of the phenomena, as to render them indistinguishable. I have had occasion to emphasize this matter in several earlier papers dealing with the subject. The observations made upon the eggs of *Pennaria* have been duplicated, almost point by point in the present case. Of course, in the egg of *Clava* there is the added di culty that all the phenomena occur within the closed gonophore. In sections, however, this fact ought to offer no serious obstructions to their detection, yet the results, as in the former, are quite as uncertain and in most cases absolutely lacking.

In the case of *Clava squamata,* Harm (*op. cit.*, p. 23) describes the phenomena in some detail, and gives almost diagrammatic drawings of the several stages. However, as will be further shown in connection with the cleavage phases, there are so many points of difference between these species that the ova may perhaps belong to very different classes so far as their character and texture are concerned. From the fact that in several particulars I have been able to confirm the observations of Harm it does not seem probable that they are so greatly different as might be implied.

I shall briefly describe the principal features which it has been possible to certainly determine, leaving others open to further inquiry or study.

As pointed out in an earlier section, about the time the ova approach full size the nuclei are to be found close to the outer periphery as will be seen in several of the accompanying photographs. It will also be observed that the eggs occupy closely the entire space of the gonophore, and that, therefore, the nuclei in coming in contact with the gonophore wall become more or

less flattened. It is at this stage that the first indications of maturation becomes apparent, namely, the shrinking and gradual disappearance of the nuclear membrane. This is particularly well shown in Fig. 2, Pl. IX. About the same time, or in some cases slightly before, there is also an evident dissolution of the chromatin network, unaccompanied by any indications of chromosomes. In this respect these eggs closely resemble those of *Eudendrium* and *Pennaria*. The most painstaking attempts to differentiate these bodies by staining operations have as frequently failed. It seems to me we are forced to the conclusion that the appearance of dissolution is indeed a fact, and that at this stage there is a general dissipation of chromatin, and perhaps other nuclear matter, into the cytoplasm.

Behavior of the Nucleolus. — Usually at about this stage marked changes take place in the nucleolus. In several instances it has been found to migrate bodily from the germinal vesicle into the cytoplasm where it is gradually dissipated and probably assimilated. At this point I find my observations closely in accord with those of Harm (*op. cit.*, p. 24). "Wahrend also hier der Nucleolus in toto vor der Polkorperchenbildung aus dem Keimblaschen heraustritt, um vom Eidotter aufgenommen und resorbirt zu werden, verbleibt er in anderen Fallen in demselben und zerfallt dort in mehrere Kugelchen."

Just prior to this migration of the nucleolus into the cytoplasm it was found to show varying degrees of vacuolation, in advanced stages of which it was often seen to partially shrink and collapse, as if there had been a loss of nucleolar substance. Something of this may be observed in several of the photographs already referred to.

Conditions very similar to these I have elsewhere described in connection with other hydroid eggs (*op. cit.*, 04[4], p. 562). Montgomery has cited many similar features which had come under his own observations, as well as observations of a like character made by many others. Those who are particularly concerned will find his discussion exceedingly interesting and suggestive, as well as including a valuable summary of evidence bearing on this problem. I may say, however, in passing that I have failed to find any indications in the present case of the metamorphosis of

the vacuolated portions of the nucleolus into yolk granules, such as Montgomery has described. On the other hand the whole of the nucleolar substance seems to be directly dissipated throughout the cytoplasm and indistinguishably assimilated by it.

Polar Bodies.—With the phenomena already described there have been found in a few cases what seemed to be polar bodies. But it was impossible to distinguish the presence of any mitotic mechanism. In every case the nuclear matter was devoid of any trace of chromatin, or, as just suggested, mitotic figures. In a few cases the formation of these bodies was observed in living eggs, and these showed essentially the same features. The nuclear matter being in close contact with the gonophore wall, small rounded portions seemed separated from the larger part, and could be distinguished close under these retaining membranes for only a short time, when they gradually disappeared, apparently resorbed, as I have suggested in former studies upon *Eudendrium* and *Pennaria.* In this respect also my observations correspond with those of Harm, though I have in no case been able to confirm his account of the phenomena of mitosis. "Die Resorption der Polkörperchen durch die eizelle sehr schnell vor sich gehen, da ich dieselben zur Zeit, wenn der weibliche Pronucleus besteht, nicht mehr habe nachweisen können."

The results in the present case, as in those of *Eudendrium* and *Pennaria*, already referred to, as well as in certain others, serve to suggest the query whether, indeed, there may not be great variation as to phenomena of maturation, even perhaps to the extent of the suppression of the more conspicuous aspects associated with it in higher forms. It may be regarded as the wildest biological heresy to even remotely suggest that in some eggs these physical phenomena might be entirely absent, but such an impression has grown upon the present writer for some time and increases with each further case, such as that under consideration. If we may have normal nuclear division without mitosis in the early embryonic history, and this I believe to be fairly beyond doubt; and if we may have differentiation without cleavage, now likewise admittedly true; and if, furthermore, we may have prior to either of these phenomena in development the dissipation of both chromatin and other nuclear matter throughout the

cytoplasm, another fact of which I can no longer doubt, then it does not seem a far call to the assumption that the reduction phenomena of maturation may well be accomplished without any of the complex and spectacular processes of mitosis. Why should the egg go through with the physical process of extruding the polar globules if they are to be immediately resorbed by the cytoplasm ?

I do not overlook the circumstance that the opacity, and in the present instance pigmentation, of the egg may establish an obstacle so formidable as to render accurate observation extremely difficult, as I have previously admitted, still the fact remains that carefully preserved and stained sections of hundreds of eggs have failed to afford convincing evidence of the presence of these bodies in distinguishable form, except in doubtful cases already referred to. It would seem somewhat remarkable that there should be all the differential results of staining upon other cell features while it should be uniformly lacking in these, usually among the most readily demonstrated.

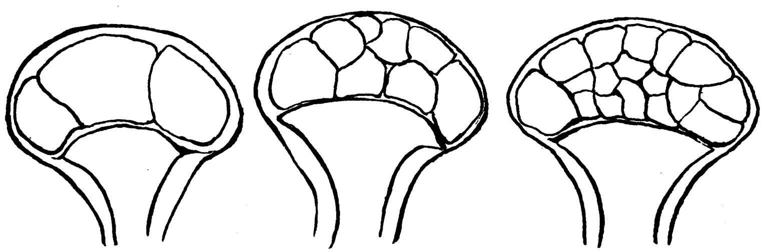
Yet another feature bearing on this point will be found in the details of cleavage, during which there is every appearance of spontaneous nuclear reorganization throughout large portions of the egg at almost the same time, the last detail of which may be followed. The facts will be described in a later section, though it seems well to call attention to their significance in relation to the matter under consideration.

CLEAVAGE.

In a general way cleavage in these eggs corresponds with that of other hydroids having similar gonophores, such as *Tubularia*, *Hydractinia*, etc. The earlier work of Ciamician ('79), and Brauer ('91), on *Tubularia mesembryanthemum*, and the later work of Allen ('00), on *T. crocea*, afford good examples of the type of cleavage here referred to. In a recent paper ('04[4]), the present writer has briefly reviewed the results of Ciamician and Brauer, and it is unnecessary to discuss here in any detail these features. Suffice it to say that Brauer found what he regarded as two rather distinct types of cleavage. The first more or less regular and equal ; the second irregular and indefinite, involving

for a time an internal nuclear proliferation, followed later by the spontaneous division of the cytoplasm into a corresponding number of blastomeres. While my own observations (*op. cit.*) did not fully confirm those of Brauer they abundantly proved the general proposition that cleavage is not uniform, either in mode or progress. The work of Allen (*op. cit.*) showed even more conclusively the variation of this feature in *T. crocea.*

The same thing is true in the eggs of *C. leptostyla.* While there is here much more uniformity than in either of the former cases the range of variation is still very considerable, as a glance at the various figures will abundantly show.



FIGS. 3, 4, 5. Camera sketches of phases of cleavage, from life.

The account of Harm (*op. cit.*, p. 28) concerning cleavage in *C. squamata* is in very marked contrast in its general aspects. Both in his descriptions and figures there is a most striking regularity and definiteness in the cleavage. He very briefly refers to a condition which he considers exceptional and abnormal, but which unless I greatly mistake must be much more common than he is disposed to think. It is quite similar to a condition referred to by Brauer, and regarded by him as likewise exceptional, but which I have showed to be more or less common.

Again, Harm describes in some detail what he regards as a more or less definite rotation of the blastomeres during earlier cleavage, in some measure comparable with phenomena familiar in the eggs of annelids and molluscs. When attention is directed to the fact that in these hydroids the eggs are usually quite closely confined within the closed walls of the gonophore, and that, whether there be two or even more in a single gonad, they are always more or less flattened against each other or against

the spadix, and that the entire course of development takes place within these walls it is difficult to see how any considerable movement of the blastomeres upon each other can be possible. Or, furthermore, how it is possible to have anything like the regulation type of cleavage characteristic of the forms just referred to, unless, perchance, the gonophores of *C. squamata* differ very greatly from those of *C. leptostyla*, which hardly seems probable. At any rate, I have not found it possible to trace any close correspondence to such features in our species, as will be seen from the following account.

As stated in an earlier connection, the gonophores of *C. leptostyla* may contain only a single egg or as many as four, though the usual number is two or three. In many cases where but two

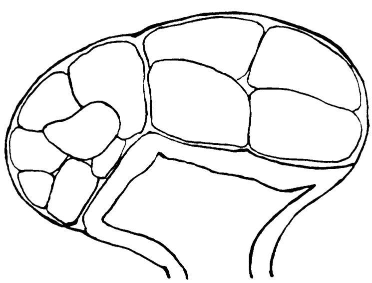


FIG. 6. Camera sketch of gonophore containing two eggs at slightly different stages.

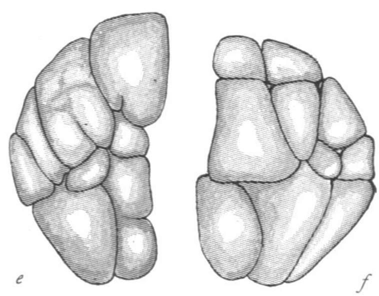


FIG. 7. Camera sketches of two eggs, *e* and *f*, as seen from side.

are found they will be upon opposite sides of the spadix, but frequently also supported side by side at the terminal portion. In either case the eggs are flattened very much on one or more surfaces, as shown in several of the figures (Fig. 7, *e* and *f*). In some cases they may even come to have a biscuit shape, or may be crescentic disks, as also shown in Figs. 3–5. In many cases as the eggs approach full growth there is a tendency to become more or less spherical, especially when occupying singly the terminal portion of the gonophore. Now, I have found that these various conditions have a more or less marked effect upon the mode of cleavage. Where the egg is spherical, for instance, cleavage is usually more or less symmetrical, as shown in Fig. 8. On the other hand, where the conditions maintain a continued

pressure the cleavage is very irregular and unequal, as shown in Figs. 6 and 7, drawn from life with the aid of the camera. In Figs. 10 to 18 are shown conditions found in sections of an egg,

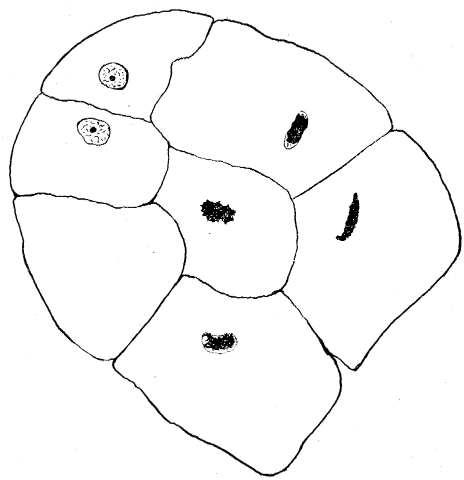


FIG. 8. Sketch of section of egg, showing nearly regular cleavage. In two cells are resting nuclei, and in others various phases of nuclear reorganization.

which are almost exactly equivalent to similar sections obtained in the study of *T. mesembryanthemum* ('04[4]).

I think it will be evident, from even a cursory study of the several figures and photographs, that the cleavage in this egg is, like that of *Pennaria*, more or less erratic and indeterminate, and conforms to none of the regular types.

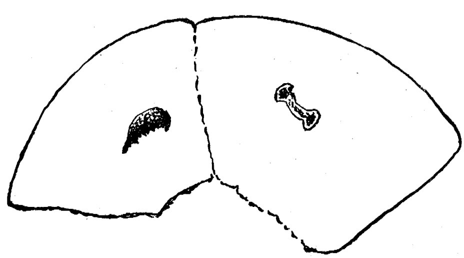
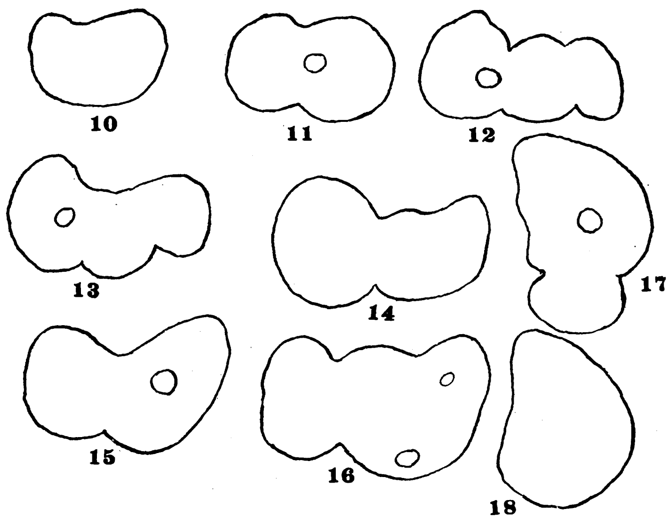


FIG. 9. Sketch of two cells of egg section, showing in one a dumb-bell shaped nucleus in amitotic division.

Another feature of cleavage remains to be considered, namely, one which involves chiefly, perhaps in some cases wholly, the

nuclei. In *Eudendrium* and in the species of *Tubularia* referred to above, it has been shown that in a considerable number of eggs there was formed by nuclear proliferation an evident syncytium, and that from this there was later a differentiation of the embryonic tissues without the process of ordinary cleavage. I have found something of the same kind in the case of *Clava*. Occasionally an egg was found among serial sections which showed no evidence of cytoplasmic cleavage, but where internal nuclear proliferation was clearly evident, and the specimens were



FIGS. 10–18. Outline sketches of sections of single egg, showing the various distribution of nuclei, shape of egg, etc.

numerous enough to enable one to definitely determine the phases of tissue differentiation and the formation of the embryo. In these cases were found the same evidences of the origin and organization of nuclei *de novo* which were found in *Eudendrium* and *Pennaria*. It was possible to trace almost every phase of this nuclear organization, from the appearance of the smallest particles of chromatin and their segregation into larger masses to the fully formed resting nucleus with its typical elements in normal relations and proportions.

Amitosis and Nuclear Organization. — During the early cleavage, even up to the sixteen-cell stage, no evidence of mitosis has

been found. I have already directed attention to its entire absence during maturation also. Now it is interesting to find abundant mitoses during the later cleavage, and during the growth of the embryo. Is it not somewhat remarkable that among the eggs of a given gonophore cluster in various stages of cleavage and later embryonic development there should be found in certain cases abundant mitoses while in others their entire absence? This cannot be attributed, under such circumstances, to differences of fixation or other details of technique, for this has been identical throughout. To what, then, may it be attributed, or how explained? As already suggested in connection with the subject of maturation, it seems to me we are forced to assume the operation of some extremely obscure bio-chemical changes which neutralize alike both acid and basic stains, or that certain phases of the mitotic mechanism may be disguised or actually lacking.

In the light of cumulative evidences along this line I think one may safely assume that the second of these alternatives is the more probable, and the facts here submitted strongly support this conclusion.

During the earlier aspects of development the nuclear phenomena associated with these eggs are extremely indefinite and obscure. During maturation there seems to be an actual fragmentation and dissipation of the nuclear matter throughout the cytoplasm. The first signs of its reorganization appear in the segregation of chromatin-like masses during the early phases of cleavage, about which the cytoplasm becomes organized into cell-like masses, as suggested in an earlier connection. At this time these chromatin masses are extremely irregular in form and size, stain very densely, and appear as indefinite, flocculent patches occupying the center of cytoplasmic aggregates, or cells. They usually assume an elongate, or often dumb-bell shaped form and seem to divide amitotically, followed by the division of the cell. In Fig. 8 is shown a camera drawing of a section of an egg, in which these features are evident. These are very common features during this period of cleavage, and must, it seems to me, be regarded as more or less typical. In two of the cells of Fig. 8 will be observed typical resting nuclei. Whether this is preliminary to the beginning of the later aspects of mitosis I am unable

to say, though it seems altogether probable. At any rate, the facts seem clearly to justify the general conclusion that for a time in the early history of the development of this egg nuclear activity differs very greatly from the ordinary forms of mitosis, and appears to involve direct, or amitotic division. During later cleavage abundant mitosis clearly indicates the prevalence of this form of cell division, if, indeed, it may not wholly supersede the other, though its appearance during regenerative activities shows the possibility of its recurrence under various conditions.

I have called attention to similar nuclear phenomena in the development of *Eudendrium* and *Pennaria*, and in the earlier paper ('04^2, p. 267), cited observations of similar sort from several sources. More recently still other cases have come to light, and it seems altogether probable that as facts multiply and attention is focused upon the phenomena cytologists will be forced to take cognizance of this form of cytogeny and give to it something more than a merely incidental place in cellular activities, and assign to it something more than senescent significance.

Among the more recent data bearing upon this point may be cited, first, the observations of Osborn ('04), in connection with the development of *Fasciolaria*; and second, similar observations by Glaser ('05), on the same organism, which go to substantially confirm the facts noted by Osborn, though with somewhat different phases of interpretation. Still a third series of facts are brought to light by Child ('04) in a paper on "Amitosis in Moniezia," in which he clearly shows the prevalence of this form of cell division in the growth of the reproductive organs and the development of the sexual cells, and expresses the belief that "future investigation will probably show that amitosis is at least as important in the life of the cell as mitosis."

As I have elsewhere pointed out, it is well known that cell division in Protozoa exhibits very different cytological features than do cleavage cells in early ontogeny.

In many, mitosis seems to be entirely lacking, while in most its features are difficult to correlate with the more typical features in metazoa. Just why we should insist upon finding among a class like cœlenterates all the details of cytogenic mechanics more or less familiar in vertebrates or other higher groups of

metazoa does not appear to be quite obvious. Moreover, why mitosis should have come to be regarded as absolutely cardinal in biologic faith is likewise uncertain. At any rate, the repeated revisions of creed as to centrosome, chromosomes, prelocalization, etc., should suggest a spirit of tolerance toward facts, whatever their significance.

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March 1, 1906.

LITERATURE CITED.

Agassiz, L.,
'62 Contr. Nat. Hist. United States, Boston. Vol. IV.

Allen, C. M.
'00 Development of Tubularia crocea, Biol. Bull., Vol. I.

Allman, J. G.
'71 A Monograph of the Gymnoblastic Hydroids, London.

Bunting, M.
'94 Origin of Sex-cells in Hydractinia, Jour. Morph., Vol. IX.

Child, C. M.
'04 Amitosis in Moniezia, Anatom. Anz., Bd. XXV.

Conklin, E. G.
'05[1] Organization and Cell-Lineage of the Ascidian Egg, Journ. Acad. Nat. Sci., Philadelphia. Vol. XIII.
'05[2] Organ-Forming Substances in Eggs of Ascidians, Biol. Bull., Vol. VIII.
'05[3] Mosaic Development in Ascidian Egg, Jour. Exp. Zool., Vol. II.

Driesch, H.
'95 Von der Entwickelung einzellner Ascidienblastomeren, Archiv. fur Entwicklm., Bd. I.

Duerdon, J. F.
'04 The Coral Siderastrea Radians and its Post-Larval Development, Carnegie Inst. Washington, 1904.

Glaser, O. G.
'05 Uber den Kannibalismus bei Fasciolaria, etc., Zeits. f. wiss. Zool., Bd. LXXX.

Haeckel, E.
'79 Das System der Medusen, Jena, 1878.

Hargitt, C. W.
'04[1] Some Unsolved Problems of Organic Adaptation, Science, Vol. XIX.
'04[2] The Early Development of Eudendrium, Zool. Jahrb., Bd. XX.
'04[3] The Early Development of Pennaria, Arch. f. Entwick., Bd. XVIII.
'04[4] Some Hydromedusae from the Bay of Naples, Mitt. Zool. Sta. Neapel, Bd. XVI.

Harm, Carl.
'02. Die Entwicklungsgeschichte von Clava squamata, Inaugural Dissertation, Leipzig. Also Zeits. f. wiss. Zool., Bd. LXXIII.

Montgomery, T. H.

'98 Cytological Studies, Jour. Morph. Vol. XV., p. 421.

Osborn, H. L.

'04 Amitosis in the Embryo of Fasciolaria, Am. Nat., Vol. XXXIII.

Weismann, A.

'83 Entstehung d. Sexualzellen bei d. Hydromedusen, Jena.

Wilson, E. B.

'03 Experiments on Cleavage Localization in the Nemertine Egg, Arch. Entwicklm. Bd. XVI.

'04 Experimental Studies on Germinal Localization, Jour. Exper. Zool. Vol. I.

Wright, T. S.

'61 Hermaphrodite Reproduction in Chrysaora hyoscella, Ann. Mag. Nat. Hist. III. ser. Vol. VII., p. 357.

Explanation of Plate IX.

Fig. 1. Photograph of section of egg under one-twelfth oil immersion, showing the crescent of pigment granules extending peripherally from the nuclear region. This area is very imperfectly shown as compared with the actual condition as seen under the microscope.

Fig. 2. Photograph of section of an egg about the period of beginning maturation. The flattened nucleus at the outer margin is evident, as is also similar change in the nucleolus. There may also be observed the general dissolution of the nucleus. Magnification as in Fig. 1.

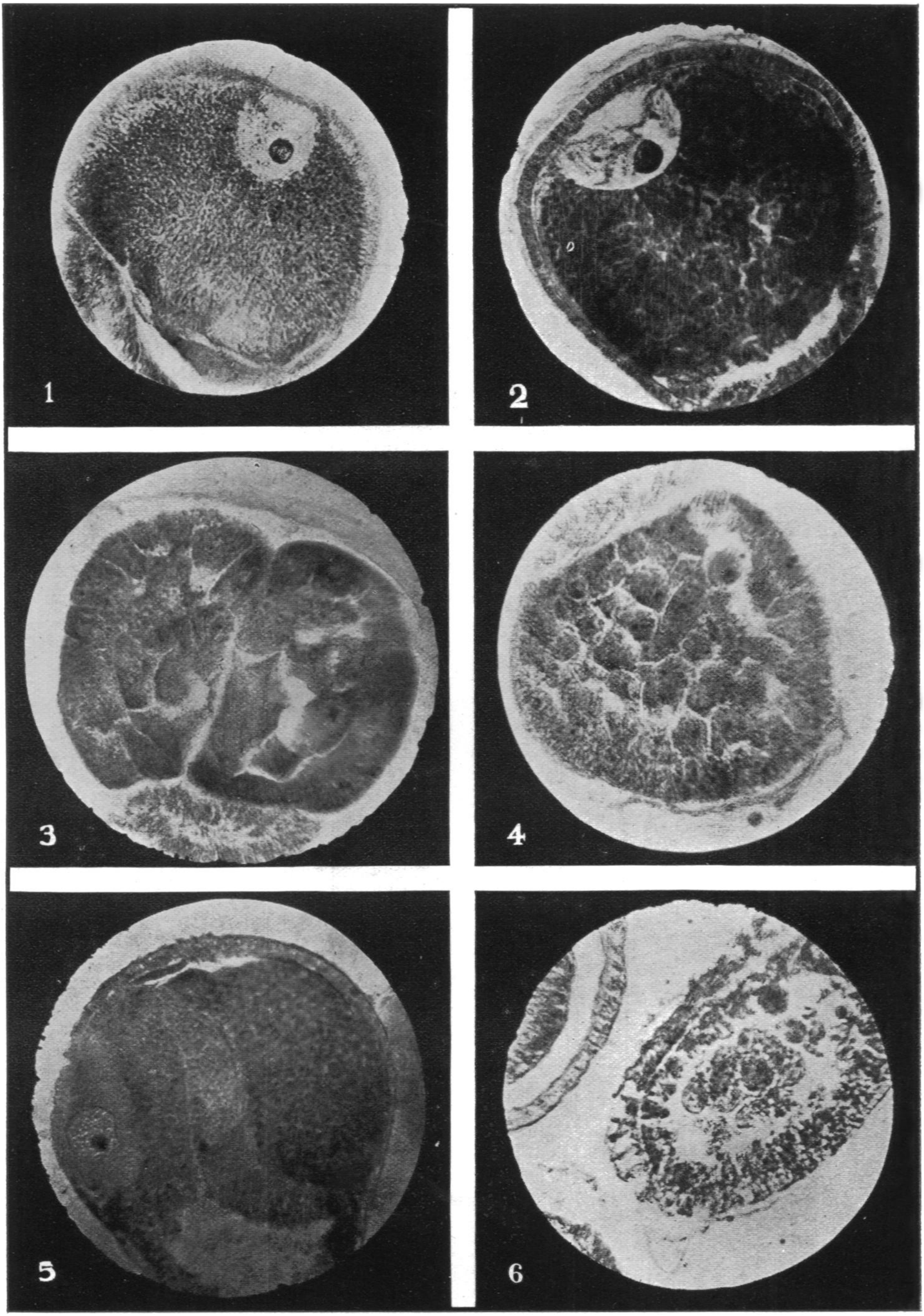
Fig. 3. Photograph of section through two eggs in a single gonophore, showing the flattening of the eggs along the line of contact. The cleavage masses may be observed as about the same stages in each egg. While this is often the case, there are exceptions, as shown in some of the text figures.

Fig. 4. Section of egg photographed under one-twelfth oil immersion, showing the more or less syncytial character of the egg at this stage of development.

Fig. 5. Section through a male gonophore, showing on one side the egg, and on the other the mass of spermatozoa, with the body of the spadix occupying the median region of the section.

Fig. 6. Photograph of portion of an embryo about ready to be liberated, showing the entoderm and ectoderm well differentiated, and with cell-like masses of pigmented yolk spheres in the enteron. It may also be observed that the ectoderm is practically free of pigment matter, as pointed out in the text.

I am under obligation to my colleague, Dr. Rogers, for the photo-micrographs illustrating the above features. All were made under the one-twelfth oil immersion lens, with arc light illumination.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